

Supporting Information for

Hit Identification and Optimization in Virtual Screening: Practical Recommendations Based Upon a Critical Literature Analysis

Tian Zhu, Shuyi Cao, Pin-Chih Su, Ram Patel, Darshan Shah, Heta B. Chokshi, Richard Szukala, Michael E. Johnson, Kirk E. Hevener**

Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, 900 S Ashland Ave., Suite 3100, Chicago, IL 60607-7173 (USA)

*To whom correspondence should be addressed.

KEH: Phone: 312-996-5388. Fax: 312-413-9303. E-mail: khevener@uic.edu

MEJ: Phone: 312-996-9114. Fax: 312-413-9303. E-mail: mjohnson@uic.edu

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Figure S1. (A) Expected relationship between percentage inhibitions at 10 μM and Log (IC_{50}) based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2). S28

Figure S2. (A) Expected relationship between percentage inhibitions at 25 μM and Log (IC_{50}) based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2)..... S29

Figure S3. (A) Expected relationship between percentage inhibitions at 50 μM and Log (IC_{50}) based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2)..... S30

Figure S4. (A) Expected relationship between percentage inhibitions at 100 μM and Log (IC_{50}) based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2)..... S31

DATA COLLECTION METHODS

1. Databases:

1.1 Web of science, Current Contents Connect, MEDLINE: 2952

Search Terms:

Topic=("virtual screen*" or "in silico screen*" or "ligand-based" or "structure-based" or "receptor-based") AND Topic=("identif*" or "discover*") NOT Topic=("review")

Refined by: General Categories=(SCIENCE TECHNOLOGY) AND Subject Areas=(BIOCHEMISTRY MOLECULAR BIOLOGY OR CHEMISTRY OR PHARMACOLOGY PHARMACY) AND Document Type=(ARTICLE OR MEETING) AND Languages=(ENGLISH)

Timespan=2007-2011.

Lemmatization=Off

1.2 PubMed: 1635-197(review)=1438

Search Terms:

((("models, chemical"[MeSH Terms] OR "models, molecular"[MeSH Terms]) OR "models, statistical"[MeSH Terms]) OR "computing methodologies"[MeSH Terms]) AND ("drug evaluation, preclinical"[MeSH Terms] OR "therapeutic uses"[MeSH Terms])) AND "drug design"[MeSH Terms] AND English[lang] AND "2007/1/1"[PDAT] : "2011/12/31"[PDAT]

1.3 Embase: 2028

Search Terms:

'virtual screen' OR 'in silico screen' OR 'ligand-based' OR 'structure-based' OR 'receptor-based' AND ('identified' OR 'identify' OR 'identification' OR 'discover' OR 'discovered' OR 'discovery') AND [2007-2011]/py AND ('Article'/it OR 'Article in Press'/it OR 'Conference Abstract'/it OR 'Conference Paper'/it)

1.4 Delete duplicates: ~4000

2. Manually filtering: ~420

3. Optimization reported in the same publication: 80

FACTORS INFLUENCING THE ACTIVITY OF INITIAL HITS

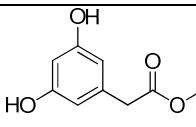
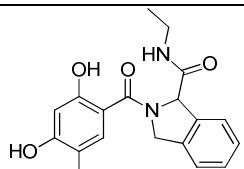
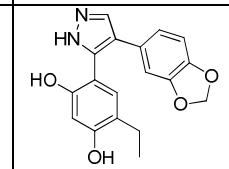
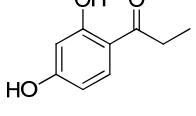
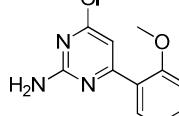
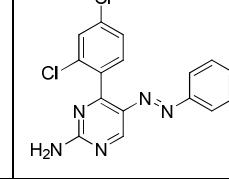
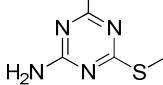
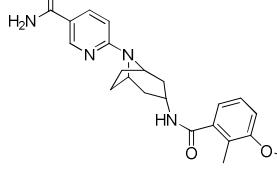
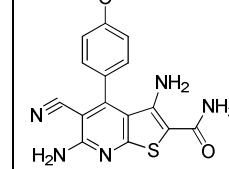
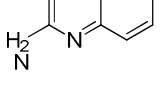
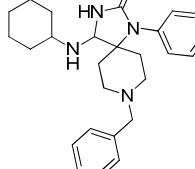
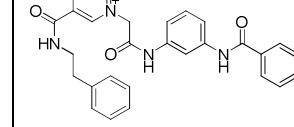
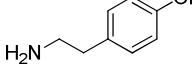
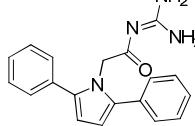
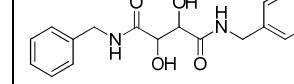
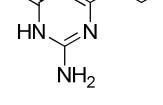
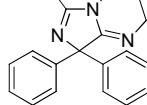
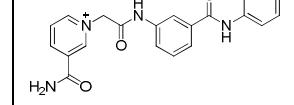
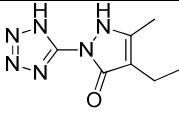
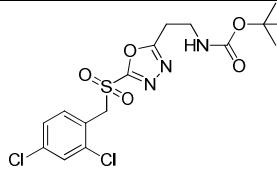
Table S1. Factors influencing the hit cutoff of initial hits.

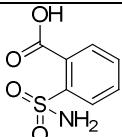
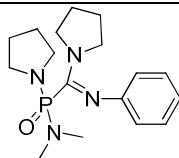
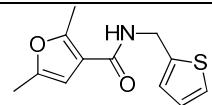
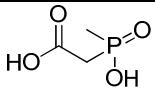
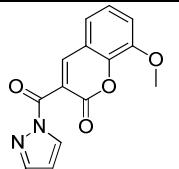
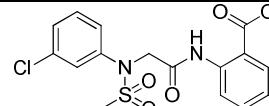
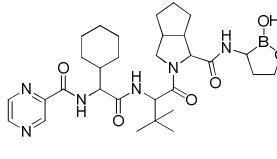
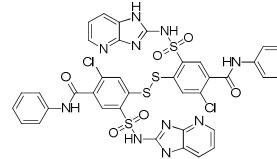
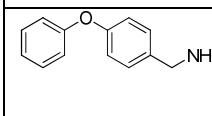
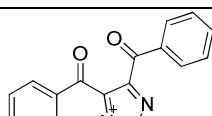
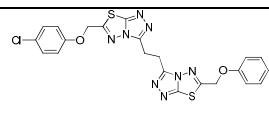
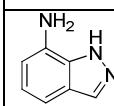
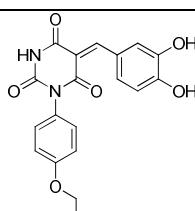
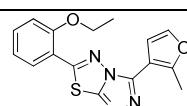
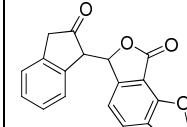
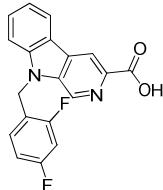
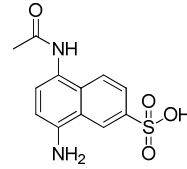
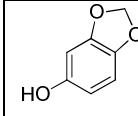
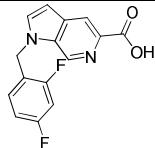
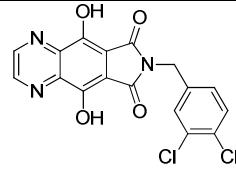
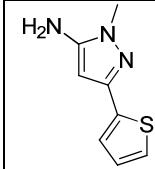
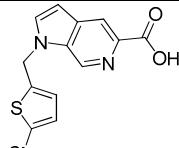
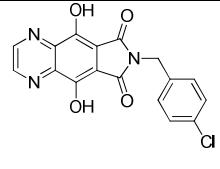
		Hit cutoff ranges (μM)					
	Total	≤ 1	1 ~ 25	25 ~ 50	50 ~ 100	100 ~ 500	> 500
VS methods							
Ligand-based	78	11	34	13	9	9	2
Structure-based	221	17	81	31	33	42	17
Ligand- & Structure-based	58	7	21	10	9	5	6
Academia versus Industry							
Academia	273	26	100	40	40	48	19
Industry	43	7	16	4	7	6	3
Academia & Industry	41	2	20	10	4	2	3
Nature of drug target							
Oxidoreductases	40	5	13	8	6	7	1
Transferases	94	11	42	10	12	15	4
Hydrolases	95	5	29	20	19	14	8
Lyases	6	1	2	1	0	2	0
Isomerases	12	0	2	2	4	3	1
Ligases	14	1	3	2	4	2	2
GPCR	22	4	13	3	0	1	1
Ion channels	8	2	4	1	1	0	0
Protein-protein interaction	5	0	3	0	0	1	1
Others	61	6	25	7	5	11	7
Journal Impact Factor							
≤ 2	15	3	7	2	1	2	0
2 ~ 3	143	13	48	23	28	21	10
3 ~ 4	54	5	22	10	1	11	5
4 ~ 5	45	5	16	6	9	6	3

5 ~ 6	85	7	36	10	11	15	6
> 6	15	2	6	3	1	1	1
Published Year							
2007	37	0	14	5	4	10	4
2008	63	10	22	8	9	11	3
2009	75	9	28	13	8	11	6
2010	91	9	33	12	17	14	6
2011	91	7	39	16	13	10	6

LE COMPARISON OF DIFFERENT SCREENING APPROACHES

Table 2. Comparison of LE values of identified hits from FBDD, HTS and VS.

Target	LE (kcal/mol/atom)					
	FBDD Hits	HTS Hits	VS Hits			
HSP90 ¹⁻⁴		0.35		0.43		0.34
		0.37		0.51		0.35
		0.47		0.29		0.33
BACE-1 ⁵⁻⁷		0.33		0.21		0.21
		0.37		0.31		0.23
		0.29		0.28		0.26
AmpC β-lactamase ^{5, 8-10}		0.25		>0.30		0.23

		0.29		0.28		0.30
		0.47		0.30		0.17
HCV NS3 ¹¹⁻¹³		0.29		0.21		0.16
		0.3		0.36		0.25
		~0.3		0.27		0.29
HIV-1 Integrase ¹⁴⁻¹⁶		0.19		0.30		0.39
		0.31		0.35		0.35
		0.29		0.39		0.35

INITIAL HITS OPTIMIZATION PAIRWISE COMPARISON

Table S3. 80 hit optimization campaigns pairwise comparison.

Title	Initial Hit			Next generation compound			LE Improved Fold	Potency Improved Fold	Method	Same Core	NHA reduced ?
	Activity	Structure	LE	Activity	Structure	LE					
Discovery of highly selective inhibitors of human fatty acid binding protein 4 (FABP4) by virtual screening	47% inhibition at 100 uM; IC50 ~100 uM	COC(=O)C1=CC(OC)=C(OC)C=C1NC(=O)CSCC(O)=O	0.24	IC50=13.5 uM	OC(=O)CSC(=O)NC1=CC(Cl)=CC(Cl)=C1	0.39	1.65	7.41	Analog search	Yes	Yes
Structure-Based Design of Potent Aromatase Inhibitors by High-Throughput Docking	IC50=59.2 nM	C1=C(C(=C(C(=C1)CC(=O)N3CC(C[N]2C=CN=C2)OCC3)OC)OC)OC	0.37	IC50=34.5 nM	C1=C(C=CC(=C1)CC(=O)N3CC(C[N]2C=CN=C2)OCC3)F	0.47	1.27	1.72	Analog search	Yes	Yes
Ligand discovery from a dopamine D3 receptor homology model and crystal structure	Ki=1.6 uM	C1(CC=C(C=C1C2=CC=C(C[N+]C(CO)(CO)C)O2)Cl)Cl	0.38	Ki=0.08 uM	C1=C(C=C(C=C1C2=CC=C(C[N+]C(CO)(CO)C)O2)Cl)Cl	0.49	1.29	20.00	Analog search	Yes	Yes
Structure-based discovery of A2A adenosine receptor ligands	Ki=0.2 uM	CC1=CC(=N)C(=N1)NC(=[N+])NCCC2=C(N)C3=C2C=C(F)C=C3C	0.38	Ki=0.4 uM	CC1=CC(=N)C(=N1)NC(=[N+])NCCC2=CC=CS2)C	0.46	1.21	0.50	Analog search	No	Yes

Ligand-based virtual screening and ADME-tox guided approach to identify triazolo-quinoxalines as folate cycle inhibitors	IC50=2.5 uM	FC(F)(F)C2=NC1=C(C=C C=C1)[N]3C(=NN=C23)C4=CC=C(Cl)C=C4	0.32	IC50=12.5 uM	C2(=NC1=C(C=CC=C1)[N]3C(=NN=C23)C4=CC=CC=C4)Cl	0.34	1.05	0.20	Analog search	Yes	Yes
Discovery of Novel Chemotypes to a G-Protein-Coupled Receptor through Ligand-Steered Homology Modeling and Structure-Based Virtual Screening	Ki=7.5 uM	CC1=CC=CC=C1SCN(CC2=CC=CC=C2)CSC3=C(C)C=CC=C3	0.26	Ki=1.7 uM	CCCCCCN(C[S](C1=CC=C(C=C1)C(=O)=O)C[S](C2=CC=C(C=C2)C(=O)=O)=O	0.27	1.05	4.41	Analog search	No	No
Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice	IC50=4.7 uM	O=C(NC1=C(C=CC=C1)C(=O)NC2CC=CCNC2=O)C3=CC=CS3	0.29	IC50=45.6 nM	O=C(NC1=C(C=CC=C1)C(=O)NC2CC=CCNC2=O)C3=CC4=C(S3)C=CC=C4	0.35	1.19	103.07	Analog search	No	No
Structure-based optimization of FDA-approved drug methylene blue as a c-myc G-quadruplex DNA stabilizer	IC50=6 uM	CN(C)C3=C2=[S+]C1=CC(=CC=C1N=C2C=C3)N(C)C.[I-]	0.34	IC50= 1 uM	C(N(C)C3=C2=[S+]C1=CC(=CC=C1N=C2C=C3)N(CC4=CC=C(C=C4)Br)C)CC5=CC=C(C=C5)Br.[I-]	0.23	0.67	6.00	Synthetic SAR	No	No

Novel Lead Structures for p38 MAP Kinase via FieldScreen	69 % inhibition at 10 uM, IC50~5 uM	COC4=CC=C(OCC3=N[N]1C(=NN=C1C2=CC=CC=C2Cl)S3)C=C4	NA	IC50=0.4 4 uM	COC1=CC=C(C=C1)CC4=N[N]2C(=N=N=C2C3=CC=CC=C3Cl)S4	0.36	NA	11.36	Synthetic SAR	No	Yes
Structure-Based Discovery of Triphenylmethane Derivatives as Inhibitors of Hepatitis C Virus Helicase	IC50=40 uM	OS(=O)(=O)C1=CC=C(C=C1)N=C1C=CC(C=C1)=C(C1=CC=C(NC2=CC(=C=C2)S(O)(=O)=O)C=C1)C1=CC=CC(=C2)S(O)(=O)=O)C=C1	0.12	IC50=10. 1 uM	C1(=C(C=CC(=C1)C(C)(C)C2=CC=C(C=C2)C(C3=CC(=C=C3)BrO)(C)C)C4=CC(=C(C=C4)OBr)OBr	0.20	1.69	3.96	Analog search and Synthetic SAR	No	Yes
Discovery and Optimization of a Novel Series of N-Arylamide Oxadiazoles as Potent, Highly Selective and Orally Bioavailable Cannabinoid Receptor 2 (CB2) Agonists	EC50=93 nM	[H]N(C=O)CCC1=NC(=NO1)C1=C(F)C=CC=C1)C1=CC=C2N(CC)C3=C(C=CC=C3)C2=C1	0.30	EC50=0. 2 nM	CC(C)(C)C1=CC(=NC(=O)CCC2=NC(=NO2)C2=CC=C(F)C=C2Cl)=NO1	0.44	1.47	465.00	Synthetic SAR	No	Yes
Identification of novel inhibitors of bacterial surface enzyme Staphylococcus aureus Sortase A	IC50=75 uM	OC(=O)C1=C(C=CC(NC(=O)C=CC2=CC=CS2)=C1)N1CCOCC	0.23	IC50=58 uM	COC(=O)C1=C(C=CC(NC(=O)C=CC2=CC=CO2)=C1)N1CCO	0.22	0.99	1.29	Synthetic SAR	No	No

		1			CC1						
Discovery of Novel Nitrobenzothiazole Inhibitors for Mycobacterium tuberculosis ATP Phosphoribosyl Transferase (HisG) through Virtual Screening	IC50=6 uM	[O-] [N+](=O)C1=CC2=C(C=C1)N=C(NC(=O)CC(NC(=O)C1=C(C=CC=C1)C1=CC=CC=C1)S2	0.22	IC50=5.5 uM	[O-] [N+](=O)C1=CC(=C(Br)C(=C1)C(=O)NNC(=O)NC1=CC=CC=C1)[N+](O)=O	0.28	1.25	1.09	Analog search	No	No
Structure-Based Drug Design of Novel Aurora Kinase A Inhibitors: Structural Basis for Potency and Specificity	IC50=15. 1 uM	CCOC(=O)C1=C([N]N=C1)NN=CC1=CC=C(OC)C=C1	0.29	IC50=33 nM	COC1=CC=C(C=NNC2=C(C(=O)NC3=CC(NC(C)=O)=CC=C3)C(C)=N[N]2)C=C1	0.33	1.15	457.58	Synthetic SAR	No	No
Identification and hit-to-lead exploration of a novel series of histamine H4 receptor inverse agonists	IC50=19 nM	CN1CCN(CC1)C1=NC=N C2=C1OC1=CC=C(Cl)C=C21	0.50	IC50=1 nM	CNC1CCN(C1)C1=NC(N)=NC2=C1O C1=C2C=C(Cl)C=C1	0.56	1.11	19.00	Synthetic SAR	No	No

Quinolone 3-Carboxylic Acid Pharmacophore: Design of Second Generation HIV-1 Integrase Inhibitors	IC50=5 uM	COC1=C(OC C2=C(Cl)C= CC=C2)C=C(C=C2C(C)= NN(C2=O)C 2=NN=N[N] 2)C=C1	0.24	IC50=4 uM	COC1=CC(C =C2C(C)=N N(C3=NN= N[N]3)C2= O)=CC(Cl)= C1OC	0.31	1.27	1.25	Analog search	No	Yes
Pharmacophore-Based Discovery of Small-Molecule Inhibitors of Protein-Protein Interactions between HIV-1 Integrase and Cellular Cofactor LEDGF/p75	46% inhibitio n at 100 uM, IC50~10 0 uM	OC(=O)C(O) =CC(=O)C1 =CN(CC2=C C=C(F)C=C2)C2=C1C=C C=C2	0.22	IC50=35 uM	OC(=O)C(O) =CC(=O)C1 =C[N]C2=C 1C(O)=CC= C2	0.34	1.55	2.86	Synthetic SAR	No	Yes
Pyrazolidine-3,5-dione derivatives as potent non-steroidal agonists of farnesoid X receptor: Virtual screening, synthesis, and biological evaluation	EC50=5. 15 uM	CC1=CC(CO C2=CC(C=C 3C(=O)NN(C3=O)C3=C C=CC=C3)= CC=C2)=CC =C1	0.25	EC50=1. 86 uM	CC1=CC(CO C2=CC(C=C 3C(=O)NN(C3=O)C3=C C=C(F)C=C3)=CC=C2)=C C=C1	0.26	1.05	2.77	Synthetic SAR	Yes	No
Design and Synthesis of Small Molecule RhoA Inhibitors: A New Promising Therapy for Cardiovascular Diseases?	Kd=5.18 uM, IC50~2.5 uM	OC(=O)C=C C1=CC(NC2 =NC3=C(C= C(C=C3)[N+](O-])=O)N=C2 NC2=CC=CC (C=CC(O)=O)=C2)=CC=C 1	0.21	IC50= 1.24 uM	OC(=O)C=C C1=CC(NC2 =NC3=C(C= C(C=C3)C=C 2)=CC=C1	0.37	1.77	2.02	Synthetic SAR	No	Yes

Small molecule inhibitors of peptidoglycan synthesis targeting the lipid II precursor	IC50=59 uM	CSC1=CC2=C(C=C1)N(C1=C(Cl)C=CC=C1)C(C)=C2CCN	0.25	IC50=29 uM	CSC1=CC2=C(C=C1)N(C1=CC=C(Cl)C(Cl)=C1)C(C)=C2CCN	0.26	1.03	2.03	Analog search	Yes	No
Structure based design of heat shock protein 90 inhibitors acting as anticancer agents	EC50=4.1 uM	CC(NC1=NC(=NC2=C1C=CC=C2)N1CCNCC1)C1=CC=CC=C1	0.24	EC50=0.902 uM	COC1=CC=C(NC2=NC3=C(C=CC=C3)C(NCC3=CC=CO3)=N2)C=C1	0.32	1.32	45.45	Analog search	No	No
Selection of Evodiamine as a Novel Topoisomerase I Inhibitor by Structure-Based Virtual Screening and Hit Optimization of Evodiamine Derivatives as Antitumor Agents	EC50=29 uM	CN1C2N(CC3=C2[N]C2=C3C=CC=C2)C(=O)C2=C1C=CC=C2	0.27	EC50=0.049 uM	CN1C2N(C=CC3=C2N(C(=O)C2=CC=C(Cl)C=C2)C2=C3C=CC=C2)C(=O)C2=C1C=CC=C2	0.31	1.16	591.84	Synthetic SAR	No	No
Discovery and preliminary SARs of keto-indoles as novel indoleamine 2,3-dioxygenase (IDO) inhibitors	IC50=65 uM	O=C(CC1=C(C=CN=C1)C1=CC2=C([N]1)C=CC=C2)	0.32	IC50=24.6 uM	C1C1=CC=C(C2=C1C=C([N]2)C(=O)C1=CC=CN=C1)	0.33	1.04	2.64	Synthetic SAR	Yes	No
The discovery and initial optimisation of pyrrole-2-carboxamides as inhibitors of p38 α MAP kinase	IC50=0.2 5 uM	CC1=C(C=C(C=C1)C(=O)C1=C[N]C(=C1)C(=O)NC1=CC=CO1)	0.39	IC50=12.59 nM	CN1CCN(CC1)C1=CC=C(C(NC(=O)C2=CC(=C([N]2)C(=O)C2=C(F)C=CC=C2)=C1)	0.36	0.92	19.86	Analog search and Synthetic SAR	No	No

Virtual Screening Identification of Nonfolate Compounds, Including a CNS Drug, as Antiparasitic Agents Inhibiting Pteridine Reductase	IC50=56 00 uM	NC1=NN=C S1	0.52	IC50=22 uM	NC1=NN=C(S1)C1=CC(= O)C2=C(O1) C=CC=C2	0.38	0.73	254.55	Synthetic SAR	No	No
Virtual screening guided discovery of novel chemotypes against hepatitis C virus NS5B polymerase	IC50=55. 2 uM	OC(=O)CN1 C(=S)SC(=C C2=CC=C(C =C2)C(F)(F) F)C1=O	0.27	IC50= 7.7 uM	C1(N(C(C(S 1)=CC2=CC =CC(=C2)O C3=CC=CC= C3)=O)CC(= O)O)=S	0.28	1.06	7.17	Synthetic SAR	No	No
Virtual screening, selection and development of a benzindolone structural scaffold for inhibition of lumazine synthase	Ki=70 uM	OC(=O)CNS (=O)(=O)C1 =CC=C2NC(=O)C3=CC= CC1=C23	0.27	Ki=38 uM	OP(O)(=O) OC(CNS(=O) (=O)C1=CC =C2NC(=O) C3=CC=CC1 =C23	0.24	0.89	1.84	Synthetic SAR	Yes	No
Discovery of novel Agonists and antagonists of the free fatty acid receptor 1 (FFAR1) using virtual screening	EC50=12 .2 uM	OC(=O)CCC CN1C(=S)SC (=CC2=CC3 =C(C=CC=C 3)C=C2)C1= O	0.27	EC50=7. 6 uM	C1=CC=C(C(C1)=S)C=C2 C(=O)N(C(S 2)=S)CCCCC (O)=O	0.32	1.18	1.61	Analog search	No	Yes

Tricyclic series of heat shock protein 90 (HSP90) inhibitors part I: Discovery of tricyclic imidazo[4,5-c]pyridines as potent inhibitors of the hsp90 molecular chaperone	IC50=7.6 uM	O=C1N2CC CC(C3=NC4 =C([N]3)C=CN=C4)=C2 C2=C1C=CC =C2	0.31	IC50=30 nM	O=C(NC1C2 C=CC=CC2C 2=C(C=CC=C12)C1=NC 2=C([N]1)C=CN=C2)C1 =CC=NC2=C1C=C[N]2	0.30	0.99	253.33	Synthetic SAR	No	No
Structure-based discovery of new small molecule inhibitors of low molecular weight protein tyrosine phosphatase	Ki=50 uM	C1(=CC2=C(C=C1)NC(C 3C2C=CC3) C4=CC=CC= C4C(F)(F)F) C(=O)O	0.23	Ki=9 uM	C1(=CC2=C(C=C1)NC(C 3C2C=CC3) C4=CC=CC(=C4)Br)C(=O)O	0.30	1.33	5.56	Analog search	Yes	Yes
Identifying novel molecular structures for advanced melanoma by ligand-based virtual screening	IC50=7.6 uM	C1=C(C=CC(=C1)C2=NC (=CS2)CSC(=[N+])N)Br. [Cl-]	0.39	IC50=1.4 5 uM	C1=C(C=CC(=C1)C2=NC (=CS2)C(=N)N)Cl	0.53	1.37	5.24	Analog search	Yes	Yes
Discovery of non-glycoside sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors by ligand-based virtual screening	EC50=3. 85 uM	C1=CC=CC2 =C1N(C(N2 CC(C(C)C)C)O)=N)CC3= CC=CC=C3	0.31	EC50=4. 6 uM	C1=CC=CC2 =C1N(C(N2 CC(O)CO)C =CC=CC=C3)=N)CC4=C C=CC=C4	0.26	0.84	0.84	Analog search	No	No
Virtual screening and synthesis of quinazolines as novel JAK2 inhibitors	44.4% Inhibitio n at 20 uM, Ec50>10 0 uM	C1=C(C=CC 2=C1N=CN =C2NN3CC OCC3)OC)O C	NA	EC50=16 .729 uM	C1=C(C=CC 2=C1N=CN =C2NC3=CC =C4C(=C3)C =CC=C4)OC)OC	0.26	NA	5.98	Synthetic SAR	No	No

Computational studies to discover a new NR2B/NMDA receptor antagonist and evaluation of pharmacological profile	86% inhibitio n at 10 uM, IC50~ 1uM, ED50=59 .2 uMkg- 1	C1=CC(=CC2=C1[N](C=C2C(CN3CC(C(CC3)CC4=O)[H])OC)OCCC)C	NA	IC50=0.7 69 uM, ED50=22 .4 uMkg- 1	C1=CC=CC2=C1[N]C=C2C(CN3CCC(CC3)CC4=C)C=CC=C4)=O	0.34	NA	1.30	Synthetic SAR	Yes	Yes
Identification of natural-product-derived inhibitors of 5-lipoxygenase activity by ligand-based virtual screening	IC50=1 uM	C1(=CC(=C(C2=C1CCC(C2)C(C(N(C3=NC=CS3)[H])=O)C)C)OCCC)C	0.32	IC50=0.8 uM	C1(=CC(=C(C2=C1CCC(C2)C(C(N(C3=NC=C(S3)C([H])=O)C)C)OCCC)C	0.32	1.02	1.25	Analog search	Yes	No
The identification of neuropeptide NTS1 receptor partial agonists through a ligand-based virtual screening approach	EC50=21 5.5 nM	C1=CC=C2C(=C1)C(C3=C2C=CC=C3)COC(=O)NC(CC(C)C)C(=O)O	0.35	EC50=17 8 nM	C12=CC=CC(=C1N=CC=C2)[S](NC3=CC4=C(C=C3)[N](C=C4)CC(NC(CC(C)C)C)C(=O)=O)(=O)=O	0.26	0.75	1.21	Analog search	No	No
High-Throughput Virtual Screening Using Quantum Mechanical Probes: Discovery of Selective Kinase Inhibitors	IC50=8.4 uM	C1(=C(SC(=C1C2=CC=C(C(=C2)C)C)C)NC(CCCC([O-])=O)=O)C(N)=O	0.27	IC50=5.2 uM	C1=CC(=CC(=C1)C2=C(SC(=C2C(N)=O)NC(C3C(C4CCC3CC4)C(=O)O)=O)C)	0.24	0.90	1.62	Analog search	No	No

Virtual screening and further development of novel ALK inhibitors	IC50=0.8 5 uM	C34=C(C=C(C=C1C(NC2 =C1C=C(C= C2)[S](=O)(=O)N(C)C)= O)[N]3)CCC C4	0.32	IC50=50. 8 nM	C3(=CC(=C(C=C1C(NC2 =C1C=C(C= C2)N)=O)[N]3)NC4=CC =CC=C4)C	0.40	1.25	16.73	Synthetic SAR	No	Yes
Identification of novel ASK1 inhibitors using virtual screening	>30% inhibitio n at 50 uM	C1=NC2=C(C(=N1)N(C) C)N=C[N]2	NA	IC50=13. 3 uM	C1(=NC2=C(C(=N1)SC) N=C[N]2)N	0.56	NA	NA	Analog search	No	No
Identification of Death-Associated Protein Kinases Inhibitors Using Structure-Based Virtual screening	84% inhibitio n at 10 uM	C1=NC=CC= C1C=C2C(O C(=N2)C3= CC=CC=C3) =O	NA	100% inhibitio n at 10 uM, IC50= 225 nM	C1=NC=CC= C1C=C2C(O C(=N2)C3= CC=CC(C(=C3)[N+]([O-])=O)Cl)=O	0.40	NA	NA	Analog search	Yes	No
Identification and optimisation of a series of substituted 5-(1H-pyrazol-3-yl)-thiophene-2-hydroxamic acids as potent histone deacetylase (HDAC) inhibitors	IC50=0.7 5 uM	C1(=N[N](C (=C1)C2=CC =C(C(NO)= O)S2)C)C(F) (F)	0.44	IC50=0.0 19 uM	C1(=N[N](C (=C1)C2=CC =C(C(NO)= O)S2)CCN3 CCC4=C(C3) C=CC=C4)C(F)(F)F	0.35	0.80	39.47	Synthetic SAR	No	No

Discovery of novel fibroblast growth factor receptor 1 kinase inhibitors by structure-based virtual screening	IC50=50 uM	C1=CC(=C(C=C1C2=NC4=C(C(=O)N2)C3=C(CC(C3)C)S4)OC)OCC(=O)[O-]	0.21	IC50=1.9 uM	C12=CC=C(C=C1C4=C(S2)N=C(C3=CC=C(C(=C3)OC)OCC(=O)[O-])]NC4=O)C	0.28	1.33	26.32	Analog search and Synthetic SAR	No	No
Elaborate ligand-based modeling and subsequent synthetic exploration unveil new nanomolar Ca2+/calmodulin-dependent protein kinase II inhibitory leads	IC50=20 nM	C1=CC(=C(C=C1NC2=N=C(=C3C(=N2)C=CC(=C3)Cl)NCCC4C)CCN4C)Cl)C	0.36	IC50=15 4 nM	C1=CC(=CC=C1NC2=N=C(=NC(=N2)NCCCC)N3CCN(CC3)C)CN(C)C[N+](=O)[O-]	0.28	0.78	0.13	Synthetic SAR	No	No
Virtual screening leads to the discovery of an effective antagonist of lymphocyte function-associated antigen-1	IC50=70 uM	C1=CC(=CC=C1C(NC(=CC3=CC=C(C2=CC(=CC=C2Cl)Cl)O3)C(=O)NCC)CO)=O)OC	0.17	IC50=4.3 uM	C1=CC(=CC=C1C(NC(=CC3=CC=C(C2=CC=C(C=C2Cl)C(F)(F)F)O3)C(=O)NCCC[N]4C=NC=C4)=O)OC	0.18	1.07	16.28	Analog search and Synthetic SAR	No	No
Identification of Novel Urease Inhibitors by High-Throughput Virtual and in Vitro Screening	IC50=34. 10 uM	C1=C(C=CC(=C1)CC2=N=N=C([N]2C3=CC=C(C=C3)OC)S)Br	0.28	IC50=10. 66 uM	C1(=CC=CC(=C1)CC2=N=N=C([N]2C3=CC(=CC=C3)OC)S)Br	0.31	1.11	3.20	Synthetic SAR	Yes	No

Discovery of new cholesteryl ester transfer protein inhibitors via ligand-based pharmacophore modeling and QSAR analysis followed by synthetic exploration	IC50=6.5 uM	CCN(CC)C1=CC=C(C=C1)NC2=CC=C(C=C2)CC(=O)C3=CC=CC=C3.Cl	0.25	33.8% inhibitio n at 10 uM	C1=C(C=CC(=C1)C(C2=C C=CC(=C2)N=CC3=CC(=CC=C3)C(F)(F)=O)C4=CC=CC=C4	NA	NA	<1	Synthetic SAR	No	
Identification and characterization of a new cognitive enhancer based on inhibition of insulin-regulated aminopeptidase	Ki=5 uM	C2(=C(C(C1=C(C=C1)[N+](=O)[O-])O2)C3=CC(=CC=C3)O)C#N)N	0.32	Ki=0.02 uM	C1=CC(=CC2=C1C(C(=C(NC(C)=O)O)2)C(=O)OC)C3=CN=C4C(=C3)C=C4=C)O	0.35	1.11	250.00	Synthetic SAR	No	No
Benzopyrazine derivatives: A novel class of growth factor receptor bound protein 7 antagonists	Kd=17.1 5 uM	C1(=CC=CC=C1)C(C2=C3=C(C=C2)N=C(C(=N3)O)C(C(=O)NC4=C(C=CC=C4)C(=O)N)=O)C(C(=O)OC)=O)=O	0.16	Kd=3.06 uM	C1(=CC=CC=C1)C(C2=C3=C(C=C2)N=C(C(=N3)O)C(C(=O)NC4=C(C=CC=C4)C(=O)N)=O)C(C(=O)OC)=O)=O	0.20	1.22	5.60	Analog search	Yes	Yes

Discovery of drug-like inhibitors of an essential RNA-editing ligase in <i>Trypanosoma brucei</i>	IC50=1.9 5 uM	OC1=CC(S(=O)(O)=O)=C C2=C1C(O)=C(N=NC3=C(C=CC=C4)C4=CC=C3)C(S(=O)(O)=O)=C2	0.24	IC50=1.0 1 uM	OC1=CC(=C(C3=C1C(=C(N=NC2=CC=C(C=C2)NCC)C(=C3)[S](=O)(O)=O)O)[S](=O)(O)=O)	0.27	1.08	1.93	Analog search	No	Yes
Identification of aminoethyl pyrrolo dihydroisoquinolinones as novel poly(ADP-ribose) polymerase-1 inhibitors	IC50=40 nM	O=C1NCCC2=C3C(=CC=C12)[N]C=C3CCN	0.60	IC50=25 nM	O=C1NCCC2=C3C(=CC=C12)[N]C=C3CCNC(=O)N4CCNCC4	0.42	0.70	1.60	Synthetic SAR	No	No
Large-scale virtual screening for the identification of new <i>Helicobacter pylori</i> urease inhibitor scaffolds	IC50=60 uM	O=C1NC(CC(N1)=O)=O	0.64	IC50=41. 6 uM	C1(C(C(NC(N1)=O)=O)=CC2=CC=C(C=C2)=O	0.38	0.58	1.44	Synthetic SAR	No	No
In silico chemical library screening and experimental validation of a novel 9-aminoacridine based lead-inhibitor of human S-adenosylmethionine decarboxylase	IC50=12 uM	NC1=NC(N)=C(/N=N/C2=CC3=C(C(N)=C(C=C(O)CC)C=C4)C4=[NH+]3)C=C2)C=C1	0.24	NA	NA	NA	NA	<1	Analog search	No	Yes

Combining Hit Identification Strategies: Fragment-Based and in Silico Approaches to Orally Active 2-Aminothieno[2,3-d]pyrimidine Inhibitors of the Hsp90 Molecular Chaperone	IC50=1.5 6 uM	C1=NC(=NC (=C1NNC2= CC=CC=C2) C3=C(C=C(C =C3)Cl)Cl)N	0.35	IC50=0.0 56 uM	C1(=NC(=N C2=C1C=C(S2)C(=O)NC C)N)C3=CC(=C(C=C3Cl) Cl)OCCNCC	0.34	0.99	27.86	Synthetic SAR	No	No
Comprehensive Mechanistic Analysis of Hits from High-Throughput and Docking Screens against -Lactamase	Ki=37 uM	C1=C(C=CC 2=C1C(=O) N(C2=O)C(C (O)=O)CC3= CC=C(C=C3) O)C(=O)O	0.23	Ki=8 uM	C1=C(C=CC 2=C1C(=O) N(C2=O)C(C (O)=O)CC3= CC=CC4=C3 C=CC=C4)C(=O)O	0.24	1.03	4.63	Analog search and Synthetic SAR	No	No
Discovery and optimization of highly ligand-efficient oxytocin receptor antagonists using structure-based drug design	Ki =50.1 nM	C1=CC(=CC =C1C(=O)N(CC2CC2)CC 3CCCC3)C4 =CC=CC=C4	0.40	Ki=0.79 nM	C1=CC(=C(C =C1C(=O)N(CC2CC2)CC 3CCCC3)C) C4=CC=CC= C4	0.48	1.20	63.42	Synthetic SAR	Yes	No
Discovery and structure-activity relationship studies of indole derivatives as liver X receptor (LXR) agonists	EC50=0. 21 uM	C1=CC=CC5 =C1C=C(C2 =CC3=C(C= C2)CC(C3)N [S](C4=CC= CC=C4)(=O) =O)[N]5C(O C(C)(C)C)=O	0.26	EC50=0. 06 uM	C1=CC=CC5 =C1C=C(C2 =CC3=C(C= C2)CC(C3)N [S](C4=C(C= CC=C4)C#N) (=O)=O)[N] 5C(OC(C)(C) C)=O	0.27	1.02	3.50	Synthetic SAR	Yes	No

Identification of new inhibitors of protein kinase R guided by statistical modeling	IC50=11 uM	C1=CC=C2C (=C1)[N]C=C2C3=CC=N C(=N3)NCOC	0.36	IC50=0.1 uM	C1=CC=C2C (=C1)[N]C(=C2C3=CC=N C(=N3)NC4 =CN=CC=C4) C	0.40	1.12	110.00	Analog search	No	No
Novel N-Substituted Benzimidazolones as Potent, Selective, CNS-Penetrant, and Orally Active M1 mAChR Agonists	EC50=0.39 uM	C1=CC=CC2 =C1NC(N2C3CCN(CC3)CCCOC4=C C=C(C=C4)F)=O	0.33	EC50=3.98 nM	C1=CC(=CC2=C1NC(N2C3CCN(CC3)C4CCOCC4)=O)C	0.50	1.52	97.99	Synthetic SAR	No	Yes
Protein–protein interaction inhibition (2P2I) combining high throughput and virtual screening: Application to the HIV-1 Nef protein	Kd=1.8 uM	CC(C)(C)C1 =CC=C(C=C1)OC(=O)N C2=CC(=C(C=C2)O)C(=O))O	0.33	Kd=0.98 uM	CC(C)(C)C1 =CC=C(C=C1)OC(=O) NC2=CC(=C(C=C2)C(=O)O)	0.34	1.05	1.84	Analog search	No	No
Search for Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase Using Chemical Similarity, Molecular Docking, and MM-GB/SA Scoring	EC50>10 0 uM	C1(=CC=C(C=C1)OC)CC2 =NN=C(O2) NC3=CC=C(C=C3)C)OC	0.23	EC50=31 0 nM	C1=CC(=C(C=C1)Cl)CC2 =NN=C(O2) NC3=CC=C(C=C3)Cl)Cl	0.39	1.70	322.58	Synthetic SAR	Yes	Yes

Virtual Ligand Screening of the p300/CBP Histone Acetyltransferase: Identification of a Selective Small Molecule Inhibitor	IC50=1.6 uM	C1(=CC=C(C=C1)C(=O)O)N2C(C(C(=N2)C)=CC4=CC=C(C3=CC(=C(C=C3[N+](O-))=O)C)O4)=O	0.24	IC50=1.2 8 uM	C1(=CC=C(C=C1)[S](=O)(=O)O)N2C(C(C(=N2)C)=CC4=CC=C(C3=CC(=C(C=C3[N+](O-))=O)C)O4)=O	0.24	0.99	1.25	Synthetic SAR	Yes	No
Ligand-based modelling followed by synthetic exploration unveil novel glycogen phosphorylase inhibitory leads	IC50=4.1 uM	COCl=CC=C(C=C1)C(=O)CC(=O)C2=CC(=CC=C2)[N+](=O)[O-]	0.34	IC50=3 uM	C1=CC=CC(=C1)NC(C(=O)NC2=CC=CC=C2C)I)=O	0.36	1.07	1.37	Synthetic SAR	No	Yes
Discovery of inhibitors of the pentein superfamily protein dimethylarginine dimethylaminohydrolase (DDAH), by virtual screening and hit analysis	IC50=16. 9 uM	C1(=CC=CO1)NC(C[N]2C=C(C3=C2C=CC=C3)C=C4C(=O)NC(N(C4=O)C5=CC=CC=C5)=S)=O	0.19	IC50=2 uM	C1=CC=CC2=C1C(=C[N]2)C=C3C(=O)N(C(NC3=O)=S)C4=CC=C(C=C4)OC	0.29	1.55	8.45	Analog search	No	Yes
Structural Studies of Pterin-Based Inhibitors of Dihydropteroate Synthase	IC50=32. 4 uM	C1(C2=C(N=C(N)N1)N(CC(=N2)C(=O)O)C)=O	0.39	IC50=25. 9 uM	C1(C2=C(N=C(N)N1)N(CC(=N2)CCNC3=CC=C(C=C3)C(=O)O)=O	0.26	0.68	1.25	Analog search	No	No

A Class of 5-Benzylidene-2-phenylthiazolinones with High Potency as Direct 5-Lipoxygenase Inhibitors	IC50=0.5 uM	C1=C(C=CC(=C1)C=C3C(N=C(C2=CC=CC=C2)S3)=O)OCC	0.39	IC50=0.1 1 uM	C1=C(C=CC(=C1)C=C3C(N=C(C2=CC=C(C=C2)Cl)S3)=O)O	0.45	1.16	4.55	Analog search	Yes	Yes
Discovery of novel purine derivatives with potent and selective inhibitory activity against c-Src tyrosine kinase	IC50=2.4 2 uM	C1(=CC=CC(=C1)NC2=N C(=NC3=C2 N=C[N]3)N 4CCNCC4)C	0.34	IC50=0.0 2 uM	C1=CC(=CC(=C1[S](=O)(=O)N)NC3=C2C(=NC=N2)[N]C(=N3)N4CCOCC4	0.41	1.21	121.00	Synthetic SAR	No	No
Structure-based virtual screening of Src kinase inhibitors	IC50=30 0 nM	C4CC(C(NC1=C(C=C2C(=C1)C(=NC=N2)NC3=C2 C(=C(C=C3)F)Cl)OCCOC)=O)NC4	0.28	IC50=89 nM	C1(CCCN1)C(=O)NC2=C(C3=C(C=C2)C(=NC=N3)NC4=C(C=CC5=C4OC(O5)Cl)OCCOC	0.28	1.02	3.37	Synthetic SAR	No	No
Design, synthesis, and biological evaluation of antiviral agents targeting flavivirus envelope proteins	EC50=51 uM	C1=CC(=C(C=C1C=CC(C2=C(N=C(S2)[N]3C(=CC(=N3)C4=CC=CC=C4)C5=CC=CC=C5)C)=O)Cl)Cl	0.17	EC50=5 uM	C1=C(C(=CC(=C1CNC(C2=C(N=C(S2)[N]3C(=CC(=N3)C4=CC=CC=C4)C5=CC=CC=C5)C)=O)Cl)Cl	0.21	1.23	10.20	Synthetic SAR	No	No

Identification and synthesis of N'-(2-oxoindolin-3-ylidene)hydrazide derivatives against c-Met kinase	IC50=1.3 uM	C1=C(C=CC =C1C(=O)N C2=CC=C(C =C2)C(=O)N /N=C/3C(=O)NC4C3C=CC=C4)C	0.27	IC50=2.2 uM	C1(=CC=CC 2=C1\ C(C(N 2CN3CCOC C3)=O)=N\ NC(=O)C4=CC=C(C=C4)OC)Br	0.26	0.96	0.59	Synthetic SAR	No	No
Small molecules block the polymerization of Z (alpha)1-antitrypsin and increase the clearance of intracellular aggregates	complet ely blocked polymerization at 50 uM while effective at reducing polymerization at 20 uM	C1=C(C=C(C (=C1C)O)C) N[S](C2=C(C)SC(=C2)C)(=O)=O	0.32	complet ely blocked polymerization at 10 uM while effective at reducing polymerization at 5 uM	C1(=CC(=C(C(=C1Br)O)C)N[S](C2=CC=CC=C2)(=O)=O	0.38	1.19	4.00	Analog search	No	Yes
Identification of a new series of STAT3 inhibitors by virtual screening	IC50=74 uM	C12=CC=CC =C1N=C(C=C2C(=O)NC3=NN=C(O3)C4=CC=CO4)C5=CC=C(C=C5	0.20	IC50=55 uM	C12=CC=CC =C1N=C(C=C2C(=O)NC3=NN=C(O3)C4=CC=CO4)C5=CC=C(C=C6=C5C=CC=C6	0.18	0.91	1.35	Synthetic SAR	No	No

Discovery of Antibacterial Biotin Carboxylase Inhibitors by Virtual Screening and Fragment-Based Approaches	IC50=12.0 uM	C1=NC(=NC(=C1)C2=CS C(=N2)C)N	0.52	IC50=0.334 uM	C1(=NC(=C=C(N)(C(=N2)C)CC3=C(C=CC=C3Cl)Cl)N	0.40	0.78	35.93	Synthetic SAR	No	No
Design, synthesis, and biological evaluation of a series of novel AXL kinase inhibitors	IC50=33 uM	C1(=CC=C(C(=C1Cl)C2=C C(=NC(=N2)NC3=CC(=C(C=C3)N4C CN(CC4)CF)C5=CC=C[N]5)F	0.18	IC50=19 nM	C1=C(C(=CC=C1)NC2=C(C=NC(=N2)NC3=CC=C(C=C3)CN4C CN(CC4)C)C)I[S](N5CCC5)(=O)=O	0.29	1.58	1736.84	Synthetic SAR	No	No
Molecular modeling studies, synthesis, and biological evaluation of Plasmodium falciparum enoyl-acyl carrier protein reductase (PfENR) inhibitors	NA	C2(=NN=C(NN=CC1=C(C=CC=C1)C(F)(F)F)[N]2)CC	NA	IC50=4.6 uM	C1=CC=CC(=C1)C2=CS C(=N2)N=C C3=CC(=C(C=C3)OC)OC	0.32	NA	NA	Synthetic SAR	No	No
Toward novel HIV-1 integrase binding inhibitors: Molecular modeling, synthesis, and biological studies	IC50=16.4 uM	C1=CC=CC2=C1N=C([N]2)C(=CC3=C C=C(O3)C4=CC(=CC=C4)C(=O)O)C#N	0.19	IC50=12.02 uM	C1=CC=CC2=C1N=C([N]2)C=CC3=C C=C(O3)C4=CC(=CC=C4)[N+] (=O)[O-]	0.27	1.40	13.64	Synthetic SAR	Yes	Yes
Evaluation of the amino acid binding site of Mycobacterium tuberculosis glutamine synthetase for drug discovery	48% inhibitory at 1 mM	C1=C(C=C(C(=C1)O)[S](=O)(=O)O)NCC(=O)O		42% inhibitory at 1 mM	C1=C(C=C(C(=C1)O)[P](OCC)(=O)OCC)NCC(=O)	NA	NA	<1	Synthetic SAR	Yes	No

)O						
Discovery of novel GSK-3(beta) inhibitors with potent in vitro and in Vivo activities and excellent brain permeability using combined ligand- and structure-based virtual screening	IC50=17 nM	C1=C(C=CC(=C1)C3=NN=C(NC2=CC(=C(C=C2)C(Cl)O3)[N+](O-))=O	0.46	IC50=7 nM	C1=C(C=CC(=C1)C3=NN=C(NC2=CC(=C(C=C2)O)C)O3)[N+](=O)[O-]	0.49	1.05	2.43	Synthetic SAR	Yes	No
Identification of novel antitubulin agents by using a virtual screening approach based on a 7-point pharmacophore model of the tubulin colchi-site	IC50=20 0 nM	C3(=CC2=C(C1=CC(=C(C(=C1)OC)O)C)OC)ON=C2C=C3)Br	0.42	IC50=35. 2 nM	C1(=CC=C(C(=C1)C(C2=CC(=C(C(=C2)OC)OC)C)=O)Br)OC	0.44	1.06	5.68	Synthetic SAR	No	No
Identification of a novel NR2B-selective NMDA receptor antagonist using a virtual screening approach	IC50=2.7 uM	C1=CC=CC=C1CN2CCN(CC2)C4=NC(C(=CC3=CC=C(C=C3)O)S4)=O	0.28	IC50=1.6 5 uM	C1=CC=CC=C1CN2CCN(CC2)C4=NC(C(=CC3=C(C=C(C=C3)O)O)S4)=O	0.28	1.00	1.64	Analog search	Yes	No
Discovery of highly potent, nonsteroidal 17(beta)-hydroxysteroid dehydrogenase type 1 inhibitors by virtual high-throughput screening	IC50= 45 nM	C1=C(C=CC2=C1OC(C2=O)=CC3=C(C=CC(=C3O)OC)O	0.48	IC50=67 nM	C1=C(C=CC2=C1OC(C2=O)=CC3=C(C=C4C(=C3)OC)CO4)O	0.45	0.93	0.67	Analog search	No	No

LIGAND EFFICIENCY AS A HIT IDENTIFICATION METRIC

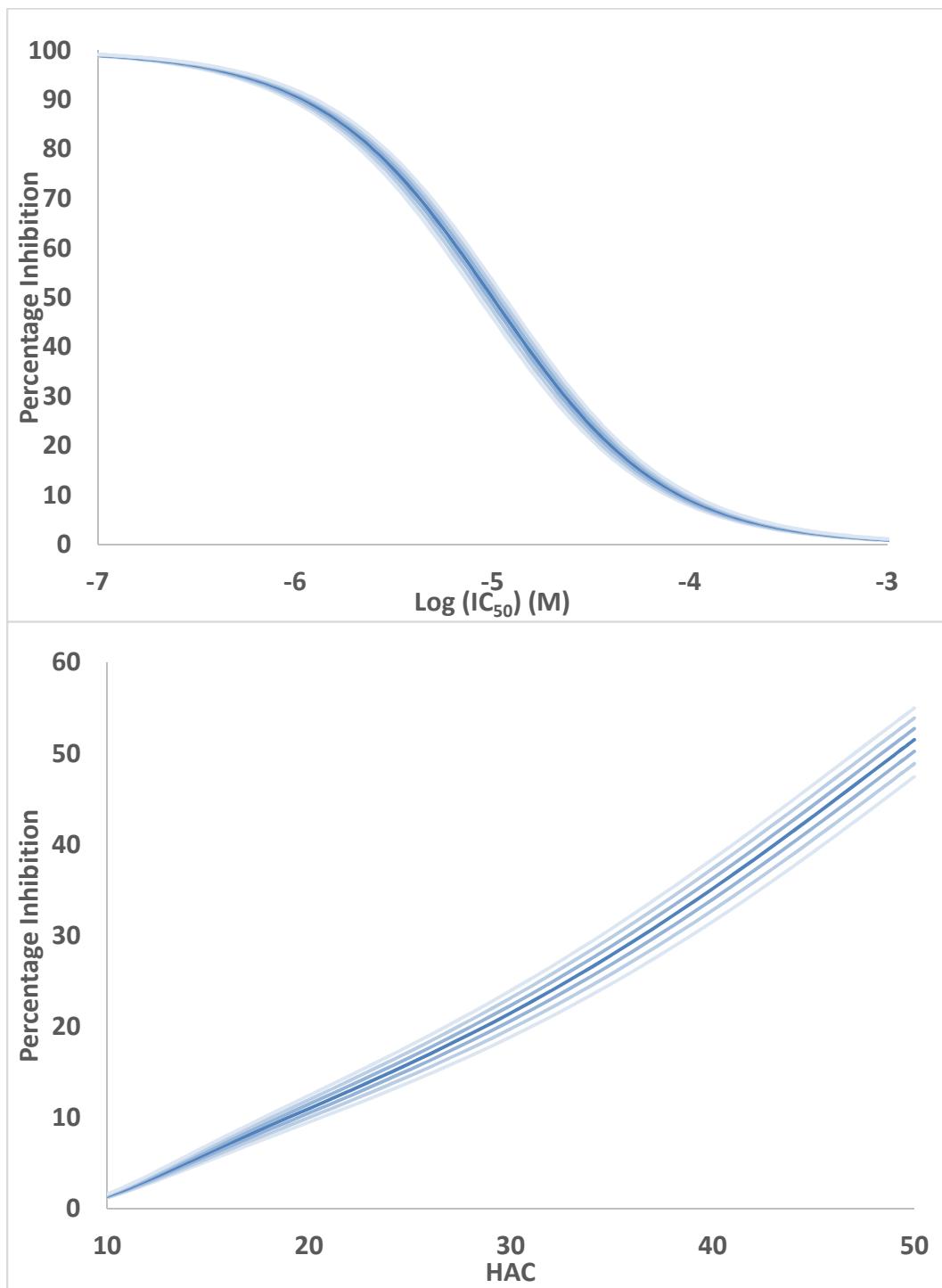


Figure S1. **(A)** Expected relationship between percentage inhibitions at 10 μM and Log (IC₅₀) based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. **(B)** Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2) $Target_{LE} = (0.467 * 1.37) / (0.0715 + \frac{7.5328}{HAC} + \frac{25.7079}{HAC^2} - \frac{361.4722}{HAC^3})$.

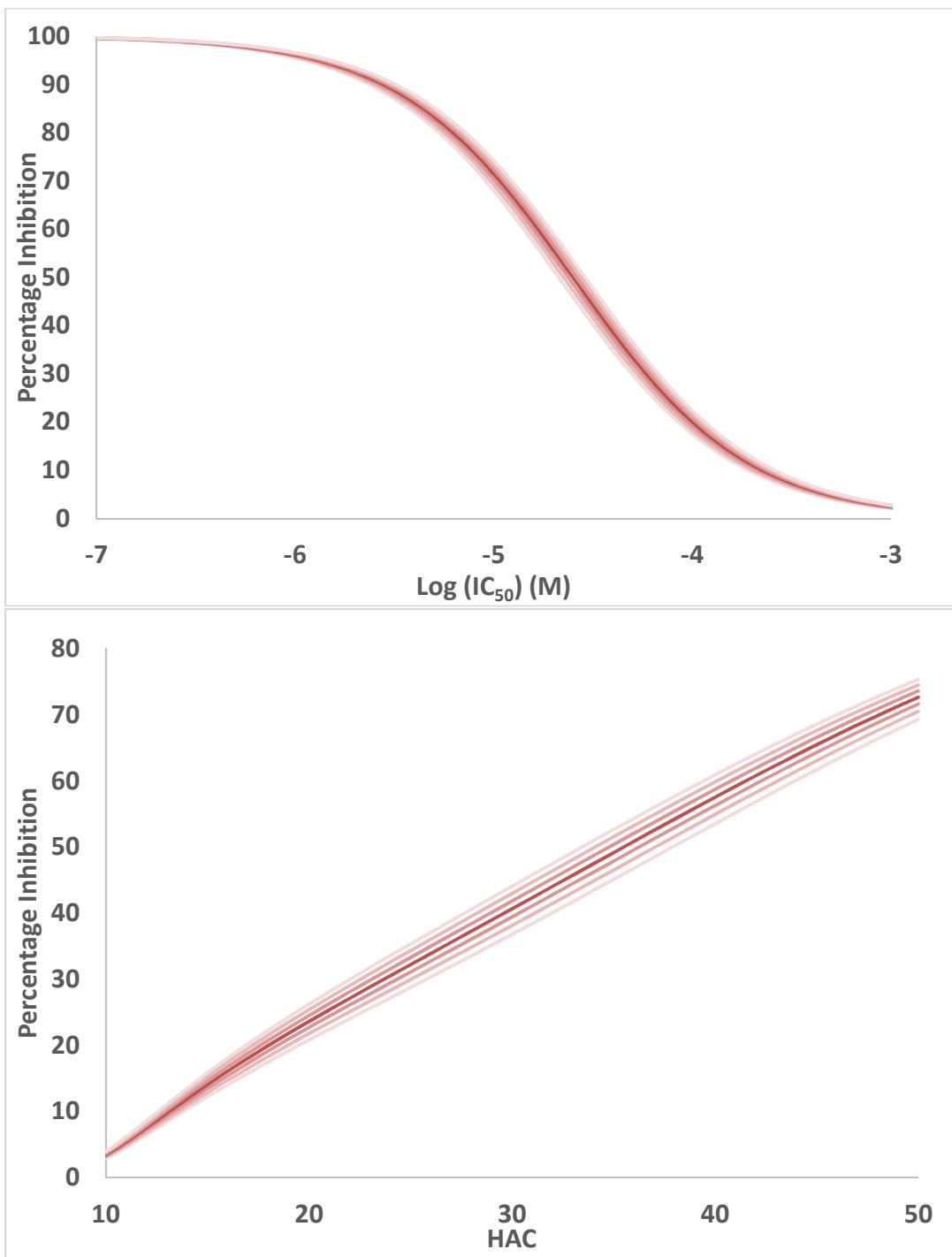


Figure S2. (A) Expected relationship between percentage inhibitions at 25 μM and $\text{Log} (\text{IC}_{50})$ based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2).

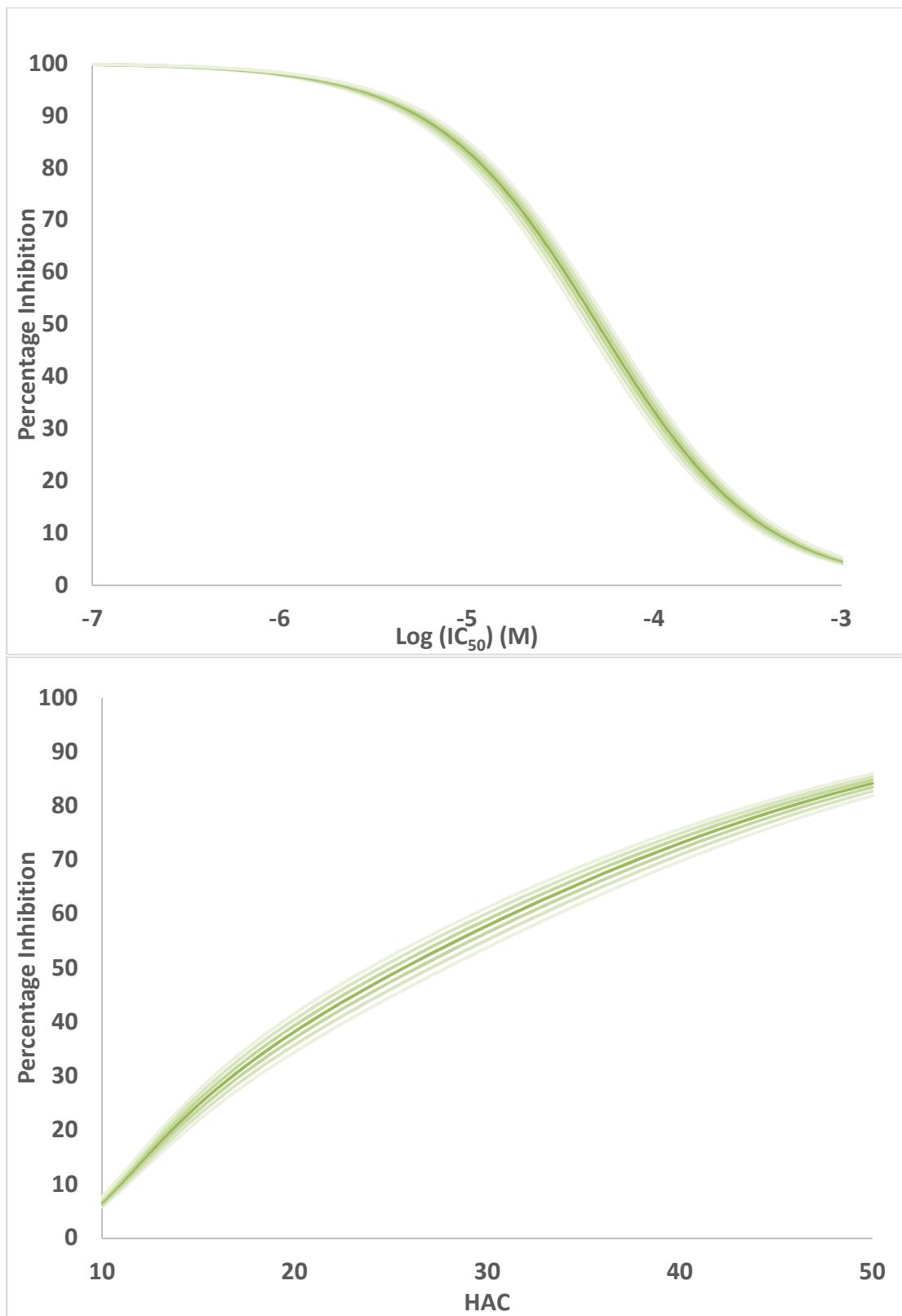


Figure S3. (A) Expected relationship between percentage inhibitions at 50 μM and $\text{Log } (\text{IC}_{50})$ based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. (B) Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2).

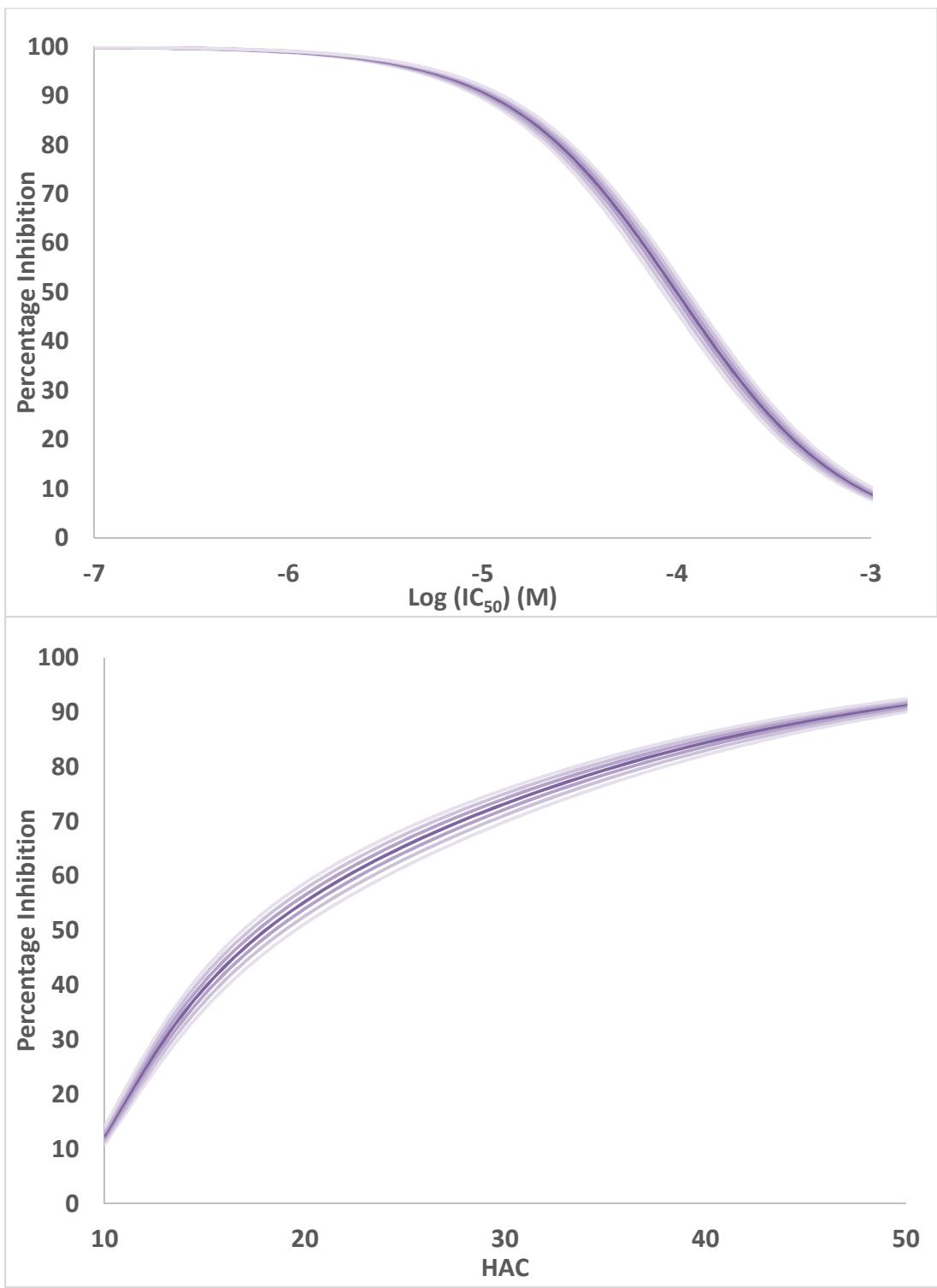


Figure S4. **(A)** Expected relationship between percentage inhibitions at 100 μM and $\text{Log} (\text{IC}_{50})$ based on the Hill slope model. 5%, 10% and 15% concentration jitter was added to show the influence of typical liquid-handling uncertainties. **(B)** Estimated percentage inhibition values at different HAC for hit selection cut-offs in order to maintain LE at target values according to Equation (2).

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